CHEMICALS MEETING THE CRITERIA FOR LISTING AS DEVELOPMENTAL AND REPRODUCTIVE TOXICANTS (DARTs) VIA THE AUTHORITATIVE BODIES MECHANISM: FOUR CHEMICALS IDENTIFIED BY U.S. EPA

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The four chemicals listed in the table below meet the criteria for listing under Proposition 65 via the authoritative bodies listing mechanism. The regulatory guidance for listing by this mechanism is set forth in Title 22, California Code of Regulations (CCR), Section 12306. For example, the regulations include provisions covering the criteria for evaluating the documentation and scientific findings by the authoritative body to determine whether listing under Proposition 65 is required.

The U.S. Environmental Protection Agency (U.S. EPA) has been identified as an authoritative body for purposes of Proposition 65 (22 CCR Section 12306(l)) and has identified the chemicals in the table below as causing developmental or reproductive toxicity. This was done by that Agency in implementing its Toxics Release Inventory (TRI) program (*i.e.*, Section 313 of the Emergency Planning and Community Right-to-Know Act of 1986 [EPCRA]). On the basis of identifying chemicals that caused reproductive, developmental and/or other toxicities the U.S. EPA added a number of chemicals to the TRI list. The U.S. EPA published its toxicity findings in the *Federal Register* (**59:**1788-1859, 1994 and **59:**61432-61485, 1994). In proposing specific chemicals for addition to the TRI list, the Agency stated that a hazard assessment was performed for each candidate, "…in accordance with relevant EPA guidelines for each adverse human health or environmental effect…" (*Federal Register* **59:**1790).

OEHHA has found that the chemicals in the table below have been “formally identified” as causing reproductive toxicity according to the regulations covering this issue (22 CCR 12306[d]) because the chemicals have “been identified as causing … reproductive toxicity by the authoritative body” (*i.e.*, U.S. EPA) “in a document that indicates that such identification is a final action” (*i.e.*, the TRI *Final Rule* [*Federal Register* **59:**61432]) and have “been included on a list of chemicals causing … reproductive toxicity issued by the authoritative body” “and the document specifically and accurately identifies the chemical” and has been “published by the authoritative body in a publication, such as, but not limited to the federal register…”

OEHHA also finds that the criteria for “as causing reproductive toxicity” given in regulation (22 CCR 12306[g]) have been satisfied for the chemicals in the table below. In making this evaluation, OEHHA relied upon the documents and reports cited by U.S. EPA in making their finding that the specified chemicals cause reproductive toxicity. In some cases,

OEHHA consulted additional sources of information on the specific studies cited by U.S. EPA. This was done only where necessary to affirm or clarify details of results and study design for studies cited by U.S. EPA; OEHHA did not review additional studies not relied on by U.S. EPA.

A major source of information used by the U.S. EPA was the "Tox-Oneliner" database maintained by U.S. EPA's Office of Pesticide Programs (OPP). This database consists of brief summaries of (usually unpublished) data submitted to the Agency in compliance with regulatory requirements. Many database entries include a notation of "core grade" – a system formerly used by U.S. EPA to indicate the extent to which a study conformed to published test guidelines (U.S. EPA 1983a and 1983b). Under this scheme, a "core grade guideline" study was considered to meet all guideline requirements; a "core grade minimum" study was considered sufficient for risk assessment; and a "core grade supplementary" study was considered to provide useful supplementary information, but not to be suitable for risk assessment on its own.

Chemical	**CAS No.**	**Endpoints**	**Pesticide status or usage**
Bromacil lithium salt	53404-19-6	developmental toxicity	Registered in CA
Bromoxynil octanoate	1689-99-2	developmental toxicity	Registered in CA
Terbacil	5902-51-2	developmental toxicity	Not currently registered in CA
Thiophanate-methyl	23564-05-8	male reproductive toxicity, female reproductive toxicity	Registered in CA

Studies cited by U.S. EPA in making findings with regard to reproductive toxicity are briefly described below. The statements in bold reflect data and conclusions which appear to satisfy the criteria for sufficiency of evidence for reproductive toxicity in regulation (22 CCR 12306[g]). Where a notation of "not stated" has been made, OEHHA staff were unable to find an explicit statement of a particular detail such as the number of animals in each dose group. Where NOELs (no-observed-effect-level), LOELs (lowest-observed-effect-level), or LELs (lowest-effect-level) are included in this document, they are quoted directly from the cited references.

Bromacil lithium salt (CAS No. 53404-19-6)

***Developmental toxicity* has been manifested as morphological abnormalities.**

U.S. EPA (1994a and 1994b) concluded that: "…there is sufficient evidence for listing bromacil lithium salt on EPCRA section 313 pursuant to EPCRA section 313(d)(2)(B) based on the available developmental …toxicity data."

In supporting the decision to add bromacil lithium salt to the TRI list, U.S. EPA (1994a) notes that, "Bromacil lithium salt will dissociated into bromacil, which is soluble in aqueous systems, and lithium ion." Therefore, developmental toxicity studies on other lithium salts, such as lithium chloride, are considered an appropriate basis for determining the hazard posed by bromacil lithium salt.

Supporting documentation for the TRI listing (U.S. EPA, 1993a) states, "Defects of the palate[,] eye and external ear were reported in the offspring of rats administered 50 mg lithium chloride intraperitoneally on gestation days 1, 4, 7, and 9 followed by 20 mg/day until day 17th [sic] (6 [Shepard, 1992]). Cleft palates were also observed in mouse fetuses when mothers were gavaged with 300 to 465 mg/kg/day lithium carbonate on gestation days 6 to 15. An increase in Esbtein's [sic] anomaly was reported among offspring of women taking lithium. Cardiovascular defects were found in 212 offspring exposed in utero to lithium therapy (6 [Shepard, 1992])."

U.S. EPA's (1993a) summary of the human data is misleading in implying that 212 cases of cardiovascular defects were reported among lithium-exposed infants. In fact, Weinstein (1979) reports on a series of 212 lithium-exposed infants, 17 of whom had cardiovascular defects. Of these 17, there were 6 cases of Ebstein's anomaly, 10 of other major cardiovascular abnormalities, and one case of an abnormal umbilical artery. According to the author, among the general population, the ratio of congenital heart disease to all nontrivial malformations is about 1:8. Among the infants on the Register of Lithium Babies, however, the ratio of congenital heart disease to all non-trivial malformations was 1:1.4. The author concluded, "We cannot say with certainty that congenital cardiovascular anomalies occur more often in lithium-exposed infants than in the non-exposed, but it is likely that they do."

With regard to the studies cited as supporting U.S. EPA's action in adding a chemical to the EPCRA-TRI list, OEHHA finds that the evidence for DART effects appears to meet the criteria of 22 CCR 12306, and notes the following:

1. **Adequacy of the experimental design:**
   Study a) rat developmental toxicity study (Wright et al., 1971). The low numbers of animals used and the use of a single dose of lithium render this study unsuitable for risk assessment purposes. However, the high frequency of lithium-induced malformations observed in this study are indicative of hazard.

Study b) mouse developmental toxicity study (Szabo, 1970). Two experiments are reported in this study. Neither experiment alone is suitable for risk assessment purposes. Taken together, the results of these experiments were consistent and indicative of hazard.
Study c) human case reports (Nora et al., 1974).
Study d) retrospective epidemilogical study (Weinstein, 1979).

2. **Route of administration:**
   Study a) ip injection.
   Study b) oral, gavage.
   Study c) not stated, presumably oral.
   Study d) not stated, presumably oral.
3. **The frequency and duration of exposure:**
   Study a) beginning on gestation day 1, 4, 7, or 9, daily through day 16.
   Study b) daily on each of gestation days 6 - 15.
   Study c) throughout at least the first trimester of pregnancy.
   Study d) throughout at least the first trimester of pregnancy.
4. **The numbers of test animals:**
   Study a) 3 pregnant animals in each test group, 3 sham controls, and 6 untreated controls.
   Study b) 1) 3 - 4 pregnant animals per dose group. 2) 15 - 20 pregnant animals per dose group.
   Study c) not relevant - paper presents two case reports of babies having Ebstein's anomaly being born to mothers undergoing lithium therapy.
   Study d) Series of 212 infants on the "Register of Lithium Babies".
5. **The choice of species:**
   Rats and mice are standard species used in toxicological studies.
6. **The choice of dosage levels:**
   Study a) the dose chosen was considered to be the maximum sublethal dosage. Initial dose was 50 mg LiCl/rat (approximately 213 mg/kg bw), subsequent doses were 20 mg LiCl/rat (approximately 85 mg/kg bw).
   Study b) 1) 200, 300, 465 mg lithium carbonate/kg bw. 2) 0, 200, 465 mg lithium carbonate/kg bw.
   Study c) therapeutic dosages of lithium.
   Study d) therapeutic dosages of lithium.
7. **Maternal toxicity:**
   Study a) not relevant - as lithium was known to be an appetite suppressant, any animals losing weight were given caloric supplementation.
   Study b) 1) 37% maternal death at the high dose of 465 mg lithium carbonate/ kg bw.
   Study c) not relevant.
   Study d) not relevant.

Bromoxynil octanoate (CAS No. 1689-99-2)

***Developmental toxicity* has been manifested in offspring as morphological abnormalities.**

The US Environmental Protection Agency (U.S. EPA, 1994a and 1994b) concluded that: "…there is sufficient evidence for listing bromoxynil octanoate on EPCRA section 313 pursuant to EPCRA section 313(d)(2)(B) based on the developmental toxicity data for bromoxynil and bromoxynil octanoate."

Supporting documentation for the TRI listing (U.S. EPA, 1993a) states, "In a dermal teratology study, bromoxynil octanoate was teratogenic to rat fetuses… Teratogenic effects (hydrocephalus, micropthalmia, anopthalmia and severe defects in ossification of the skull) were observed in rabbits administered 60 mg/kg/day bromoxynil by gavage… Fetotoxicity (increases in all forms of supernumerary ribs) was observed in rats at 5 mg/kg/day… several other developmental studies indicate potential developmental toxicity of bromoxynil phenol."

With regard to the studies cited as supporting U.S. EPA's action in adding a chemical to the EPCRA-TRI list, OEHHA finds that the evidence for DART effects appears to meet the criteria of 22 CCR 12306, and notes the following:

1. **Adequacy of the experimental design:**
   Study a) rat dermal teratology study. Rated core grade supplemental, "pending submission of additional information" (U.S. EPA, 1994c).
   Study b) rabbit teratology study on bromoxynil, rated core grade guideline (U.S. EPA, 1997).
   Study c) rat teratology study - No core grade reported in U.S. EPA documents consulted.
2. **Route of administration:**
   Study a) dermal.
   Study b) oral, gavage.
   Study c) not stated in U.S. EPA documents consulted.
3. **The frequency and duration of exposure:**
   Study a) daily on each of gestation days 6 - 15.
   Study b) not stated directly, but as this study was rated "core grade guideline" it would have met U.S. EPA test guidelines (U.S. EPA, 1983a) which require exposure on gestation days 6 - 15.
   Study c) not stated.
4. **The numbers of test animals:**
   Study a) 25 animals per dose group (U.S. EPA, 1989).
   Study b) not stated directly, but as this study was rated "core grade guideline" it would have met U.S. EPA test guidelines (U.S. EPA, 1983a) which require a minimum of 12 rabbits per dose group.
   Study c) not stated.
5. **The choice of species:**

The rat and rabbit are standard species used in toxicological studies.

6. **The choice of dosage levels:**
   Study a) 0, 2, 5, 10, 15, 20, and 75 mg/kg/6hrs/day (U.S. EPA, 1989).
   Study b) 0, 30, and 60 mg/kg/day. As this study was rated core grade guideline, it would have met U.S. EPA test-guideline standards (U.S. EPA, 1983a), which require a minimum of three doses. Hence there was presumably at least one other dose-level tested.
   Study c) 0, 1.5, 5, 30 mg/kg/day.
7. **Maternal toxicity:**
   Study a) The maternal LOAEL, based on reduced body weight gain, was 20 mg/kg/day, with a corresponding NOAEL of 15 mg/kg/day. The LOAEL and NOAEL for developmental toxicity in this study were 15 and 10 mg/kg/day, respectively.
   Study b) IRIS (U.S. EPA, 1997) describes 30 mg/kg/day as both the NOEL and the LEL for maternal animals in this study. The endpoint of maternal toxicity is stated to be body weight loss. The developmental LEL, for major malformations, in this study was 60 mg/kg/day, with a corresponding NOEL of 30 mg/kg/day.
   Study c) the maternal LOEL, based on body weight loss, was 30 mg/kg/day. Developmental toxicity was observed at 5 mg/kg/day, with a NOEL of 1.5 mg/kg/day.

<u>Terbacil (CAS No. 5902-51-2)</u>

***Developmental toxicity* was manifested as reduced viability in offspring of exposed rats.**

U.S. EPA (1994a and 1994b) concluded that "…there is sufficient evidence for listing terbacil on EPCRA section 313 pursuant to EPCRA section 313(d)(2)(B) based on the available … developmental toxicity data for this chemical."

Supporting documentation for the TRI listing (U.S. EPA, 1993a) states, "Decreases in the number of implantations and live fetuses, were observed in rats administered 62.5 mg/kg/day (LOEL) orally on days 6-15 of gestation. The NOEL was 12.5 mg/kg/day (24 [U.S. EPA, 1994c]). Significantly reduced body weights were observed in the offspring of rabbits orally administered 600 mg/kg/day (LOEL) orally on days 6-15 [sic] of gestation. The NOEL was 200 mg/kg/day (24 [U.S. EPA, 1994c], 11 [U.S. EPA , 1993b]). The studies were classified Core Minimum."

Review of the original data cited by U.S. EPA revealed that excessive maternal toxicity (mortality rate of 39%) was associated with findings of reduced fetal weight in the rabbit teratology study. Therefore, this study should not be used to make a listing determination.

With regard to the studies cited as supporting U.S. EPA's action in adding a chemical to the EPCRA-TRI list, OEHHA finds that the evidence for DART effects appears to meet the criteria of 22 CCR 12306, and notes the following:

1. **Adequacy of the experimental design:**
   Study a) rat developmental toxicity study - classified core grade minimum.
   Study b) rabbit developmental toxicity study - classified core grade minimum. OEHHA considers this study to be invalid, due to excessive maternal mortality.
2. **Route of administration:**
   Study a) oral.
   Study b) oral.
3. **The frequency and duration of exposure:**
   Study a) daily on gestation days 6 - 15.
   Study b) daily on gestation days 7 - 19 (U.S. EPA, 1988).
4. **The numbers of test animals:**
   Study a) not stated, but study was considered to meet guideline requirements, which specify a minimum of 20 pregnant rats per dose group (U.S. EPA, 1983a).
   Study b) 18 animals per dose group (U.S. EPA, 1988).
5. **The choice of species:**
   Rats and rabbits are standard species for developmental toxicity testing.
6. **The choice of dosage levels:**
   Study a) 0, 250, 1250, 5000 ppm.
   Study b) 0, 30, 200, or 600 mg/kg/day.
7. **Maternal toxicity:**
   Study a) maternal LEL = 1250 ppm for reduced body weight, maternal NOEL = 250 ppm.
   Study b) maternal LEL = 600 mg/kg/day for increased mortality, decreased weight gain, and clinical signs of toxicity; maternal NOEL = 200 mg/kg/day. At the LOEL dose for developmental toxicity (600 mg/kg/day), maternal mortality reached a frequency of 39%.

<u>Thiophanate-methyl (CAS No. 23564-05-8)</u>

***Male reproductive toxicity* was evidenced by decreased spermatogenesis in rats. *Female reproductive toxicity* was manifested as reduced numbers of implantations in mice, and reduced litter weights in rats.**
U.S. EPA (1994a and 1994b) concluded that "…there is sufficient evidence for listing thiophanate-methyl on EPCRA section 313 pursuant to EPCRA section 313(d)(2)(B) based on the reproductive toxicity data for this chemical."

Supporting documentation for the TRI listing (U.S. EPA, 1993a) states, "Decreased spermatogenesis was observed in male rats fed diets containing 32 mg/kg/day thiophanate-

methyl; the NOEL was 8mg/kg/day (IRIS, 1993 [U.S. EPA, 1993b]). Based on the NOEL for decreased spermatogenesis, an oral RfD of 0.08 mg/kg/day was derived (IRIS, 1993 [U.S. EPA, 1993b]); the confidence in the study, database, and RfD was rated high. ... In a 3-generation reproductive study in rats, reduced litter weights were seen at a daily dietary dose of 32 mg/kg thiophanate-methyl. The NOEL was 8 mg/kg/day. A decrease in the number of implantations was observed in mice administered a limit dose of 1,000 mg/kg/day."

With regard to the studies cited as supporting U.S. EPA's action in adding a chemical to the EPCRA-TRI list, OEHHA finds that the evidence for DART effects appears to meet the criteria of 22 CCR 12306, and notes the following:

1. **Adequacy of the experimental design:**
   Study a) 2-year rat feeding study. Rated core grade minimum. The study was used as the principal study in determining the oral RfD for thiophanate-methyl. Confidence in the study was rated as high, "The principal study appears to be of high quality and is given a high rating." (U.S. EPA, 1993b).
   Study b) 3-generation rat reproductive toxicity study. The study was graded 'core minimum' (U.S. EPA, 1993b).
   Study c) limit test. The study was rated 'core grade supplemental' (U.S. EPA, 1993b).
2. **Route of administration:**
   Study a) oral, feed.
   Study b) oral, feed.
   Study c) not stated.
3. **The frequency and duration of exposure:**
   Study a) daily in feed for 2 years.
   Study b) not stated, but U.S. EPA test guidelines for reproductive toxicity studies (U.S. EPA, 1983b) specify continuous exposure from prior to mating of the parental generation, throughout gestation and lactation, and continuing through postnatal development and reproduction of the F1 generation to produce the F2. As the study was considered to meet guideline requirements, it is presumed that this dosing schedule was adhered to. As the study was stated to involve 3 generations, dosing was presumably continued for F2 animals though production of the F3 generation.
   Study c) not stated.
4. **The numbers of test animals:**
   Study a) 35 males and 35 females per dose group. 50 males and 50 females served as controls.
   Study b) not stated, but U.S. EPA test guidelines for reproductive toxicity studies (U.S. EPA 1983b) specify sufficient animals to ensure a minimum of 20 pregnant animals per dose group. As the study received an acceptable grade, it is presumed that guideline requirements were met.
   Study c) not stated.
5. **The choice of species:**

Rats and mice are standard species for reproductive toxicity testing.

6. **The choice of dosage levels:**

   Study a) 0, 10, 40, 160, or 640 ppm.

   Study b) 0, 8, 32 mg/kg/day. As the study was rated 'core grade minimum' (U.S. EPA, 1993b), there was presumably at least one additional dose level, which would have been required to meet U.S. EPA test guideline standards for a reproductive toxicity study (U.S. EPA, 1983b).

   Study c) 1000 mg/kg/day.

7. **Maternal toxicity:**

   Study a) not relevant.

   Study b) not stated.

   Study c) not stated.

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